# THE EFFECT OF FOOD CONTENT AND TEMPERATURE ON RESPIRATION IN PELOMYXA CAROLINENSIS WILSON [1]

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## Introduction

*Pelomyxa carolinensis* Wilson (*Chaos chaos* Schaeffer) is an organism favorable for the study of cellular functions. It is one of the largest of the amoeboid forms, it can be cultured easily in the laboratory, and it is relatively simple in structure and physiological activity. Although it has been available from stock culture since 1937, apparently no studies have been made on the respiration of this organism; in fact, very few investigations have been made on respiration in any of the amoeboid forms.

Emerson (1929), using Barcroft-Warburg manometers, found that the oxygen consumption of *Amoeba proteus* at 20° C. amounts to 0.16 mm.$^3$ per mm.$^3$ of cell substance per hour. Howland and Bernstein (1931), using a modification of the capillary tube method devised by Kalmus (1928), found that the oxygen consumption of *Actinosphaerium eichhornii* at 20° C. is of the order of 1,100 mm.$^3$ per hour per million organisms. They did not express the rate of oxygen consumption per unit volume of protoplasm.

The following investigations were carried out to ascertain the rate of respiration in *Pelomyxa carolinensis* under various conditions of nutrition and at different temperatures.

## Material and Methods

The organisms used in these experiments were from a strain which has been cultured in the laboratory for several years in the manner described by Belda (1942). The pelomyxae were grown in Hahnert's (1932) solution, but prior to each experiment the organisms were kept for about a week in a culture medium buffered to maintain a hydrogen-ion concentration of pH 6.8. To keep this value constant, a high concentration of phosphates was required. Pelomyxae, put into experimental solutions containing only potassium and magnesium compounds, quickly disintegrated. However, the addition of calcium chloride served to counteract the toxic effects of the potassium ions, and the pelomyxae grew well in this medium. The formula which was finally adopted is given in Table I.

The pelomyxae were kept in glass finger bowls containing 150 ml. of buffered solution. Food was supplied for the organisms by adding portions of a centrifuged culture of *Paramecium caudatum* until there were about 600 to 700 paramecia per ml. Under these conditions the pelomyxae ingest from one to three paramecia

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TABLE I

*Buffered culture solution for* Pelomyxa carolinensis

| | |
|---|---|
| $K_2HPO_4$ | 80 mg. |
| $KH_2PO_4$ | 80 mg. |
| $CaCl_2$ | 104 mg. |
| $Mg_3(PO_4)_2 \cdot 4H_2O$ | 2 mg. |
| $H_2O$ (redistilled) | 1,000 ml. |

within an hour, and continue feeding as long as any paramecia remain. Specimens of Pelomyxa were removed from the finger bowls at intervals for respiration studies.

The rate of oxygen consumption and carbon dioxide elimination was measured by means of a Barcroft-Warburg apparatus. The shaking rack held seven manometers and flasks, of which one was used as a thermo-barometer. Preliminary tests had shown that there was no measurable difference in the rate of oxygen consumption between pelomyxae tested in flasks which contained 100, 200, or 300 organisms. In the experiments as carried out, groups of either 100 or 200 specimens were used in each test.

To measure the rate of oxygen consumption, 0.4 ml. of 10 per cent KOH was put into the inset and 0.3 ml. of 3N HCl into the onset of each flask. To measure both the rate of oxygen consumption and of carbon dioxide elimination, the manometers were paired and 0.4 ml. of distilled water was put into the insets of half of the flasks and 0.4 ml. of 10 per cent KOH into those of the other half. The water bath of the apparatus was kept at the temperature selected for each experiment with a variation of not more than $\pm$ 0.05° C.

The manometers were mounted on a shaking mechanism which was operated at the rate of 124 complete cycles per minute through an amplitude of 3 cm. This amount of motion provided a sufficiently rapid exchange between the gases and liquids in the flasks.

After the manometers and flasks had been put into place with the stopcocks open, the shaking mechanism was run for one hour. At the highest and lowest temperatures used in the experiments, this period was extended to 2 hours. By this time the temperature in the flasks was equal to that of the water bath and practically all of the carbon dioxide originally present in the flasks had been absorbed. The stopcocks were then closed, and the level of the liquid in each manometer was recorded at intervals of one hour.

The volume of the pelomyxae was calculated by measuring specimens in a volumescope. This apparatus, devised by Chalkley (1929) and modified by Belda (1942), consists essentially of a capillary pyrex glass tube into which a pelomyxa can be put. The bore of the tube is such that the pelomyxa assumes a cylindrical shape with rounded ends. The length of the specimen can be measured accurately by means of a compound microscope provided with a camera lucida. The volume of the specimen can be calculated by using the equation

$$V = \pi r^2 l + 4/3\pi r^3$$

in which $r$ is the radius, both of the cylindrical part of the pelomyxa and of each of the rounded ends, and $l$ is the length of the cylindrical part.

The surface area of a pelomyxa varies considerably, depending on the number of pseudopodia which may be extended. The average surface area is, however, approximately equal to that of a specimen which has the shape of an elongated cylinder (Belda, 1943). Accordingly, the equation

$$A = 2\pi rl + 4\pi r^2$$

in which the symbols have the same value as in the preceding equation, is appropriate for calculating the approximate surface area.

In conducting the experiments, 20 specimens of average size were selected from those to be used in each test. These specimens were taken up with a capillary pipette and measured in the volumescope. At the conclusion of each test 20 specimens were again selected and measured. From these measurements the average volume and surface area was calculated. There was no appreciable variation between the average values obtained at the beginning and at the end of each experiment.

## Results

### *I. Effect of food content on respiration*

In much of the work previously reported on cellular respiration in the Protozoa, the food content of the organisms was not considered. However, Lund (1918) observed that there was a decrease in the rate of oxygen consumption in paramecia which had been starved for 20 hours, and Leichsenring (1925) observed a decrease of 23 per cent in the rate of oxygen consumption in paramecia which had been starved for 24 hours.

In order to ascertain what types of pelomyxae would be suitable for studying the effect of different temperatures on the rate of respiration, preliminary experiments were carried out on specimens in varying states of nutrition. The first tests were made with what are designated well-fed specimens. About 1000 pelomyxae together with numerous paramecia were put into finger-bowls containing buffer solution as described under Material and Methods. After 24 hours each pelomyxa had several large food vacuoles containing partly digested paramecia. Several hundred pelomyxae of average size were removed with a capillary pipette and washed in three separate portions of sterile buffer solution. Five milliliters of sterile buffer solution containing either 100 or 200 pelomyxae were put into each manometer flask; and 5 milliliters buffer solution without pelomyxae were put into the flask of the thermo-barometer. In the first three tests both oxygen consumption and carbon dioxide elimination were measured. The results are presented in Table II.

In the first three tests, the rate of oxygen consumption was 15,530 mm.$^3$, and of carbon dioxide elimination 13,510 mm.$^3$, per hour per million organisms. Calculated from these values, the respiratory quotient is 0.87.

For the second part of the experiment, pelomyxae were put into finger bowls containing buffer solution without paramecia and left for one week without food. These are designated starved pelomyxae. At the end of this time all food vacuoles have disappeared, and the average volume of the specimens is about 25 per cent less than that of well-fed pelomyxae. The average rate of oxygen consumption of

starved pelomyxae per million organisms was found to be about 65 per cent less than that of well-fed pelomyxae; however, the rate of oxygen consumption per mm.$^3$ of cell substance showed a decrease of only 54 per cent. In the first three tests with starved pelomyxae, the average rate of carbon dioxide elimination per million organisms was 3,490 mm.$^3$ per hour, a decrease of 73 per cent from that of well-fed specimens. The respiratory quotient was 0.56.

In the third part of the experiment, pelomyxae were put into finger bowls containing buffer solution, and large numbers of paramecia were added as in the first part of the experiments. After 3 days the pelomyxae had ingested nearly all the paramecia, but still contained numerous large food vacuoles. The pelomyxae were

TABLE II

*Rate of oxygen consumption in starved, "normal," and well-fed specimens of* Pelomyxa carolinensis
Temperature: 25° C. Hydrogen-ion concentration: pH 6.8

| Type of organism | Test number | Number of organisms in each test | Duration of experiment | Average volume of one million organisms in mm.$^3$ | Average rate of $O_2$ consumption in mm.$^3$ per hour per million organisms | Average rate of $O_2$ consumption in mm.$^3$ per hour per mm.$^3$ of cell substance |
|---|---|---|---|---|---|---|
| Starved | 1 to 3 | 100 | 5 hours | 27,600 | 6,200 | 0.188±0.052 |
| | 4 to 6 | 200 | 6 hours | | 4,890 | |
| | 7 to 9 | 200 | 6 hours | | 4,340 | |
| | | | | | Mean: 5,210±1,450 | |
| "Normal" | 1 to 3 | 100 | 5 hours | 35,580 | 9,730 | 0.275±0.032 |
| | 4 to 6 | 200 | 7 hours | | 9,950 | |
| | 7 to 9 | 200 | 12 hours | | 9,730 | |
| | | | | | Mean: 9,800±1,140 | |
| Well-fed | 1 to 3 | 200 | 5 hours | 36,800 | 15,530 | 0.407±0.040 |
| | 4 to 6 | 200 | 7 hours | | 15,270 | |
| | 7 to 9 | 100 | 7 hours | | 14,120 | |
| | | | | | Mean: 14,970±1,500 | |

left in the finger bowls for 3 more days, but no more paramecia were added. At the end of this time most of the organisms still had two or three small food vacuoles. These organisms are designated "normal" pelomyxae. The results of all these experiments are combined in Table II. This table shows that the rate of respiration in *Pelomyxa carolinensis* is closely correlated with the amount of food material present in the cytoplasm, for there is a progressive increase in the rate of oxygen consumption in starved, "normal," and well-fed specimens.

### *II. Effect of temperature on respiration*

In the previous experiment, there was less variation in the rate of oxygen consumption of "normal" pelomyxae than of starved or well-fed specimens. Accordingly the remaining tests were all made with "normal" specimens.

A number of tests were made to measure the rate of oxygen consumption of

Pelomyxa at temperatures between 15° C. and 40° C., using 5° increments. Groups of either 100 or 200 organisms in 5 ml. buffer solution, treated as described above for "normal" pelomyxae, were tested. The results are given in Table III. This table shows that the rate of oxygen consumption for Peolmyxa increases in nearly

TABLE III

*Rate of oxygen consumption in* Pelomyxa carolinensis *at different temperatures. Hydrogen-ion concentration, pH 6.8*

Each figure under oxygen consumption per million organisms represents the average for three tests except those taken at 25° C. which are averages for four tests.

| Temperature | Test number | Number of organisms used | Duration of experiment | Average volume of one million organisms in $mm.^3$ | Average approximate surface area/ million organisms in $mm.^2$ | Average rate of $O_2$ consumption in $mm.^3$ per hour per million organisms | Average rate of $O_2$ consumption in $mm.^3$ per hour per $mm.^3$ cell substance |
|---|---|---|---|---|---|---|---|
| 15° C. | 1 to 3 | 200 | 7 hours | 34,950 | 583,000 | 4,660 | 0.144±0.024 |
| | 4 to 6 | 200 | 7 hours | | | 5,080 | |
| | 7 to 9 | 100 | 4 hours | | | 5,280 | |
| | 10 to 12 | 200 | 5 hours | | | 5,130 | |
| | | | | | | Mean = 5,040± 850 | |
| 20° C. | 1 to 3 | 200 | 7 hours | 36,800 | 607,900 | 7,070 | 0.191±0.022 |
| | 4 to 6 | 200 | 5 hours | | | 6,920 | |
| | 7 to 9 | 100 | 5 hours | | | 7,150 | |
| | | | | | | Mean = 7,050± 827 | |
| 25° C. | 1 to 4 | 100 | 12 hours | 36,800 | 607,900 | 10,250 | 0.244±0.028 |
| | 5 to 8 | 200 | 10 hours | | | 8,750 | |
| | 9 to 12 | 200 | 6 hours | | | 8,170 | |
| | 13 to 16 | 100 | 12 hours | | | 8,540 | |
| | 17 to 20 | 200 | 10 hours | | | 9,930 | |
| | 21 to 24 | 200 | 6 hours | | | 8,380 | |
| | | | | | | Mean = 9,010± 910 | |
| 30° C. | 1 to 3 | 200 | 12 hours | 35,580 | 590,000 | 10,990 | 0.372±0.049 |
| | 4 to 6 | 100 | 12 hours | | | 14,455 | |
| | 7 to 9 | 200 | 5 hours | | | 14,420 | |
| | | | | | | Mean = 13,244±1,760 | |
| 35° C. | 1 to 3 | 150 | 5 hours | 34,950 | 583,000 | 19,010 | 0.507±0.044 |
| | 4 to 6 | 200 | 6 hours | | | 17,530 | |
| | 7 to 9 | 100 | 6 hours | | | 16,630 | |
| | | | | | | Mean = 17,749±1,540 | |

linear ratio from 15° to 25° C. Between 25° and 35° C. the rate of oxygen consumption increases more rapidly, but again in nearly linear ratio.

The lowest temperature at which tests were carried out was 15° C. At this temperature the pelomyxae showed a tendency to become spherical. The amount of protoplasmic streaming and the rate of locomotion were considerably diminished.

At the higher temperatures, the pelomyxae were more active. At 35° C. the rate of locomotion was rapid; long, narrow pseudopodia were extended in many directions, so that the organisms were nearly stellate in shape. All the specimens remained alive during these tests.

In nine of the 12 tests which were made at 40° C. the pelomyxae died during the first hour. In the remaining three tests, the organisms survived long enough for a single reading of the manometers to be made. The average rate of oxygen consumption for the three tests, each made with 100 pelomyxae at 40° C., was 28,300 mm.$^3$ per hour per million organisms. This rate is much higher than that at 35° C. Because of the high mortality of the organisms at 40° C., the results obtained are probably not comparable to those at lower temperatures, and they are not included in the table.

The temperature coefficient ($Q_{10}$) for the rate of oxygen consumption of Pelomyxa between 15° and 25° C. is 1.7; between 25° and 35° C. the $Q_{10}$ is 2.1.

## Discussion

The rate of oxygen consumption in well-fed pelomyxae is 2.16 times that of starved specimens. This difference is similar to that found by Lund (1918) in comparable types of Paramecium. He found that specimens tested after having been fed boiled yeast suspension used up from two to three times as much oxygen as specimens tested after having been starved.

The rate of oxygen consumption of pelomyxae kept for 7 days without food is 32 per cent less than that of "normal" specimens kept for 3 days with practically no food. Similarly, the rate of oxygen consumption of pelomyxae kept for 3 days with practically no food is 32 per cent less than that of well-fed pelomyxae. This difference is comparable to a decrease of 29 per cent in paramecia after having been kept for 72 hours without food, as measured by Leichsenring (1925). The close agreement between the results for Paramecium and Pelomyxa may, however, not be significant, since the two organisms differ greatly in their structure and activity.

The published data for respiration in Paramecium are based on the quantity of oxygen consumed and carbon dioxide given off per organism. They do not take into account the volume nor the surface area of the specimens. At least approximate values for the volume and surface area of Paramecium can be calculated by means of the equations given by Fortner (1925). These, however, require measurements of the length and diameter of the specimens.

That the volume of the organisms ought to be considered, at least in tests made on organisms differing in food content, can be seen from Table II. The decrease in the rate of oxygen consumption per unit of protoplasm for starved pelomyxae is relatively less than the decrease per organism.

Apparently the only measurement of the respiratory quotient of an amoebid organism previously reported is that of Emerson (1929), who found a value of slightly less than 1.0 for *Amoeba proteus*. However, the R.Q. for other free-living Protozoa has been measured. Emerson (1929) found a value of slightly less than 1.0 for *Blepharisma undulans*. The R.Q. for Paramecium, as found by various investigators, is as follows: Amberson (1928), 0.69; Root (1930), 0.62; and Mast, Pace, and Mast (1936), 0.72. These values are all similar to that of

*Pelomyxa carolinensis.* The significance of measurements of the R.Q. for various Protozoa has been discussed at length by Jahn (1941).

The rate of oxygen consumption per hour per mm.$^3$ of protoplasm for Pelomyxa at 20° C., namely 0.191 mm.$^3$, is slightly higher than the rate of 0.16 mm.$^3$ for *Amoeba proteus* found by Emerson (1929). Considering possible errors in the measurements of the volume of the organisms, this difference may not be significant. The rate of oxygen absorption per unit of surface area in *Amoeba proteus* is, however, much less than that in Pelomyxa. An exact comparison is not possible because Emerson did not measure the surface area of Amoeba. But since the volume of Amoeba is only about 1/50 that of Pelomyxa (Belda, 1942), the amount of surface area of Amoeba per mm.$^3$ of protoplasm is many times greater than that of Pelomyxa.

Howland and Bernstein (1931) did not calculate the rate of oxygen consumption per unit volume of protoplasm in *Actinosphaerium eichhornii.* However, an approximate value can be obtained. The average diameter of *Actinosphaerium eichhornii* is between 200 and 300 micra. Since the organisms are approximately spherical in shape, the volume can readily be calculated from the diameter. The average rate of oxygen consumption at 20° C. is 0.26 mm.$^3$ per mm.$^3$ of protoplasm for specimens of Actinosphaerium having a diameter of 200 micra, and 0.11 mm.$^3$ for specimens having a diameter of 300 micra. These values are of the same order of magnitude as those for Pelomyxa at 20° C.

The rate of oxygen consumption of Pelomyxa at 15° C. is 25 per cent less than that at 20° C. This difference is nearly equal to that observed in Paramecium by Leichsenring (1925). She measured the rate of respiration in paramecia at 20° C., then lowered the temperature to 15° C., and found that the rate of respiration had decreased 30 per cent.

Leichsenring also tested paramecia at 20° C., then increased the temperature to 35° C., and found an increase of 35 per cent in the rate of respiration. This is much less than the increase in rate found in the present investigation over the same range of temperature, since the rate of oxygen consumption in Pelomyxa is 135 per cent higher at 35° C. than at 20° C. It should be kept in mind that Leichsenring observed the effects of change in temperature on the same culture of Paramecium, whereas in the present experiment different lots of Pelomyxa were used at the different temperatures. However, Barratt (1905) found that the rate of $CO_2$ production of Paramecium at 27–30° C. was more than twice that at 15° C. This difference is nearly equal to that difference in the rate of oxygen consumption of Pelomyxa between these temperatures.

The $Q_{10}$ value of 1.7 between 15° and 25° C. for pelomyxa is less than the $Q_{10}$ value of 2.1 found by Lwoff (1933) for Paramecium between 13° and 23° C. On the other hand, the $Q_{10}$ value of 2.1 for Pelomyxa between 25° and 35° C. is higher than the value of 1.5 for Paramecium between 23° and 32° C. found by Kalmus (1928) and also by Lwoff (1933).

## Summary

1. The rate of respiration in *Peolmyxa carolinensis* at 25° C. is closely correlated with the amount of food material present in the cytoplasm.

2. The rate of oxygen consumption at 25° C. was found to be $0.244 \pm 0.028$

mm.$^3$ per hour per mm.$^3$ cell substance and does not differ greatly from that of *Amoeba proteus* and *Actinosphaerium eichhornii*. The rates were less at low temperatures (15° C.) and greater at high temperatures (35° C.).

3. Temperatures above 35° C. are usually lethal to Pelomyxa, although in several tests the oxygen consumption at 40° C. for one hour was obtained.

4. The respiratory quotient is much lower for starved specimens (R.Q.: 0.56) than for well-fed specimens (R.Q.: 0.87) and is approximately equal to that of other free-living Protozoa.

5. As the temperature is increased, the rate of oxygen consumption increases, but the rate of increase becomes progressively greater.

6. The temperature coefficient for the rate of respiration in *Pelomyxa carolinensis* is nearly the same as that in Paramecium, varying from 1.7 between 15° and 25° C. to 2.1 between 25° and 35° C.

## LITERATURE CITED


Amberson, E. F., 1929. The influence of oxygen tension upon the respiration of unicellular organisms. *Biol. Bull.*, **55**: 79–91.

Barratt, J. O. W., 1905. Die Kohlensäureproduktion von Paramecium aurelia. *Z. allg. Physiol.*, **5**: 66–72.

Belda, W. H., 1942. Permeability to water in Pelomyxa carolinensis. I. Changes in volume of Pelomyxa carolinensis in solutions of different osmotic concentration. *The Salesianum*, **37**: 68–81.

Belda, W. H., 1943. Permeability to water in Peolmyxa carolinensis. III. The permeability constant for water in Pelomyxa carolinensis. *The Salesianum*, **38**: 17–24.

Chalkley, H. W., 1929. Changes in water content in Amoeba in relation to changes in its protoplasmic structure. *Physiol. Zool.*, **2**: 535–74.

Emerson, R., 1929. Measurements of the metabolism of two Protozoans. *Jour. Gen. Physiol.*, **13**: 143–158.

Fortner, H., 1925. Über die Gesetzmässigkeit der Wirkungen des osmotischen Druckes physiologisch indifferenter Lösungen auf einzellige, tierische Organismen. *Biol. Centralbl.*, **45**: 417–446.

Hahnert, W. F., 1932. Studies on the chemical needs of Amoeba proteus: a culture method. *Biol. Bull.*, **62**: 205–211.

Howland, R. B., and A. Bernstein, 1931. A method of determining the oxygen consumption of a single cell. *Jour. Gen. Physiol.*, **14**: 339–348; 352–403.

Jahn, T. L., 1941. Chapter VI. Respiratory metabolism. *Protozoa in Biological Research*. Columbia Univ. Press.

Kalmus, H., 1928. Die Messung der Atmung, Gärung, und $CO_2$-Assimilation kleiner Organismen in der Kapilläre. *Zeit. vergl. Physiol.*, **7**: 304–313.

Leichsenring, J. M., 1925. Factors influencing the rate of oxygen consumption in unicellular organisms. *Amer. Jour. Physiol.*, **75**: 84–92.

Lund, E. J., 1918. III. Intracellular respiration, relation of the state of nutrition of Paramecium to the rate of intracellular oxidation. *Amer. Jour. Physiol.*, **47**: 167–177.

Lwoff, A., 1933. Die Bedeutung Des Blutfarbstoffes für die parasitischen Flagellaten. *Zbl. Bakt. Abt.* 1 (Orig.), **130**: 498–518.

Mast, S. O., D. M. Pace, and L. R. Mast, 1936. The effect of sulfur on the rate of respiration and on the respiratory quotient of *Chilomonas paramecium*. *Jour. Cell. and Comp. Physiol.*, **8**: 125–139.

Root, W. S., 1930. The influence of carbon dioxide upon the oxygen consumption of Paramecium and the egg of Arbacia. *Biol. Bull.*, **59**: 48–69.